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Limitations of Euglenidae as Polluted Water Indicators

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Casual inspection of many of the streams of this country gives the impression that they might be suitable for fishing, bathing, public water supplies, stock-watering, boiler water supplies or some of the myriad uses to which relatively pure waters can be put. Tracing the stream courses may reveal however that even the best looking streams get heavy doses of domestic sewage, cannery wastes, paper mill wastes, distillery wastes or other injurious substances and that such flowing waters are largely useless or even harmful to man and his cattle, empty of fish and shunned by waterfowl. Many of the streams of the Eastern United States are vastly changed from what they were two or three generations past and an awakening public interest in their decreasing economic, public health, recreational and aesthetic values is demanding that those responsible for stream abuse, whether cities, corporate interests, or private individuals, be compelled to stop such abuse, whose injurious effects touch the population at large.

Criteria of stream pollution may be all too evident in turbid, highly colored, offensive smelling streams. But often relatively clear sparkling streams need laboratory examination to determine the nature and extent of their pollution and this is a public service usually performed by city, state or federal skilled scientific personnel. Determinations of the sanitary conditions of waters are chemical, bacteriological and biological.

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Biological examinations may include several features as the microscopic life present, or the animals living in or on the bottom. For sometime it has been stated that the presence of certain microscopic organisms in abundance is a sure indication of organic pollution, usually domestic sewage. One of the

groups used in this fashion has been the "Euglenas and their allies", a related group of green flagellates belonging to the lowest plant or animal classifications, the Algae or Protozoa, but difficult to place categorically in either because the organisms include some of the characteristics of each group. Kolkwitz and Marsson (1), Marsson (2) and Fair & Whipple (3) refer to the Euglenidae as indicators of pollution in the above manner.

Euglenidae in the Scioto River

Between April, 1937, and November, 1938, the senior author examined 884 samples taken from the Scioto River of south central Ohio. Samples were taken from as many as 18 stations along the river and tributary creeks, and for the greater part of the time each point was sampled weekly. Half the samples were examined after formalin preservation and half as fresh unpreserved samples. Usually 100 ml. of river water was centrifuged until the suspended matter had been concentrated in a small amount of catch. All organisms were identified, if possible, and counted. The Scioto at times has a very large plankton content, but ample time was devoted to the critical examination and enumeration of catches so that the nature and extent of the plankton population is very accurately known.

Five genera of green Euglenidae were found comprising 48 species. In addition one group of Euglena and one of Trachelomonas were called simply "species", inasmuch as specific determinations were not possible. One group of several possible species of Trachelomonas was called T. urceolata (Fig. 1) since it comprised a large intergrading series of forms. Total numbers of each recognizable species were tabulated for each station examined on a given date, then a separate sheet was made for each species showing the date of sampling and the numbers of that species present per ml. of raw river water. This enabled one to determine almost at a glance whether that species had a seasonal or all-year distribution; how it was affected by rises in the river, and the time and place

of its maximum occurrences.

Figure 2 shows the average numbers of green Euglenidae per ml. at each station examined during these 19 months. Figure 3 shows their seasonal distribution at mile 30 (Red Bridge) approximately 23 miles below the Columbus sewage outfall. From these two charts it is at once evident that the average number of green Euglenidae is high for the 76 mile stretch from Columbus to Kilgour Bridge, and that they show a pronounced seasonal distribution.

The first sampling point, mile 3, is just downstream from a low head dam at Columbus and the river there is unpolluted, receiving no sewage and being only a few miles below the Griggs and O'Shaughnessy reservoirs. At about mile 7 it receives the discharge of the Columbus sewage treatment plant, which during 1937 and 1938 poured a poorly treated effluent into the river. During the hot weather low water stages the river was visibly foul at least as far as mile 17. Much of this stretch was a region of slow flow, through pools separated by shallow ripples, offering an excellent chance for the rapid multiplication of plankton during low water, warm weather conditions. From about mile 17 a steady improvement in the condition of the river was noticeable and this visual evidence was supported by chemical and bacteriological findings. Certainly the river was not grossly polluted at mile 30 (Red Bridge) nor did it get sufficient sewage or waste materials at any point below to markedly deteriorate the water.

The zone in which the Euglenidae were most abundant in two years was not the most foul water zone, but from mile 30 to mile 76 downstream. One sharp decrease in numbers occurred at mile 32 but there was an immediate rise and at this time there is no indication of what factor was responsible for the decrease here. In other words, the greatest numbers of green Euglenidae in the Scioto occur in the zone in which recovery from heavy pollution is taking place, and the decrease in numbers at mile 32 is a minor fluctuation.

Of course, the Scioto is a flowing stream and, because of the time element, enormous numbers of Euglenidae should not be expected at the precise point where it becomes heavily polluted by Columbus sewage, even if Euglenidae react favorably to sewage by multiplying rapidly. But des Cilleuls[4] has summarized the findings of many workers showing that plankton multiplication is rapid and most favored in still areas of streams such as bays, dead arms and the like, and that these feed a constant supply of plankton into the current. There are numerous still areas in the Scioto within the heavily polluted stretch, and examination of samples from such points failed to reveal them as "breeding places" for Euglenidae. Weston and Turner[5] long ago pointed out that a sewage polluted stream recovers biologically before it recovers chemically. At Red Bridge chemical recovery of the Scioto is generally very evident, hence biological recovery would be expected above this point. Actually, the increase of Euglenidae is slow between mile 7 and mile 23. (Figure 2) but rises sharply thereafter and remains high. If the stimulus to this high development is derived from pollution, either it is a very sustained one, or it is due to some substance or condition not shown by chemical analysis.

If we examine individual species most of them have their maximum occurrence in the recovery zone, even if their numbers are small. Some species as Cryptoglena pigra, Euglena fusca or Phacus anacoleus showed few numbers most of the time, but sometimes flared up suddenly into numbers somewhat comparable to the familiar blooms of Euglenas occasionally seen on ponds or temporary pools. Others as Euglena oxyuris or Trachelomonas volvocina were present most of the time over widely scattered mileages, but never attained large numbers. Still others as Euglena pisciformis, E. viridis, Trachelomonas crebea and T. urceolata were common in occurrence much of the time. Figure 4 shows the average distribution of E. viridis in the Scioto for the period studied. The increase in numbers of Euglena viridis in the grossly polluted zone is striking compared to the behaviour

of the Euglenida as a whole. But the increase is short lived, for there is a decrease between mile 13 and mile 23, which allows a time interval when multiplication might be possible, or expected, if sewage provides a stimulus. Furthermore, the greatest numbers of Euglena viridis are in the cleaner waters between miles 30 and 40. For this particular species the data certainly suggest a favorable reaction to waters recently foul with organic matter.

Figure 5 shows a similar reaction for the group of Trachelomonads grouped as T. urceolata. But there is an initial decrease for these organisms where pollution is heaviest, and recovery in numbers is slow, until past mile 23.

Of the individual species of Euglenidae whose total numbers and wide distribution are sufficient for critical use, E. viridis comes nearest to indicating a preference for polluted water, yet even its maximum and greatest occurrence is in the recovery zone. Figure 5 shows a much greater average occurrence of T. urceolata in the zone still further down.

If any individual set of samples is selected for analysis, false conclusions may easily be drawn. Table 1 shows a few instances of the distribution of certain species on certain dates, with maxima for the same species at one time in the zone of greatest pollution, at another time in the recovery zone. Such instances demonstrate the necessity for using average distribution figures. It has not been possible thus far to trace one of these maxima downstream. While aggregations of plankton organisms move down to some extent, it has not yet been shown whether the observed maxima move at current speed, whether they lag, or whether they are dispersed as the current carries them into a changed environment. Such information would be useful and work on this point is urgently needed.

TABLE 1

Distribution of Various Euglenidae in the Scioto River
on Certain Dates. Numbers per ml. of Raw Water

SPECIES	DATES	Sanitary Condition of Water																	
		Clean	Polluted			Transitional			Clean										
		Sampling Stations in River Miles Below Columbus, Ohio																	
		3	13	17	23	30	33	35	46	61	67	76	92	120	(a)	(b)	(c)	(d)	(e)	(f)
Euglena fusca	6-20-38		2	26	16	14		14	46	6					2					
" "	7-27-38			1					18	2	8	4								
" pisciformis	8-5-37	2	42	34	14	40	2	16		4										
" "	8-30-38	4	1	1	4	110		30	1	2	1					1				
" polymorpha	8-5-37	18	64	38	68	28														
" "	8-30-38	8	18	21	265		256	44	12	11					4	34	9			
" viridis	8-18-37		34	50	32	44	4	42		10										
" "	8-30-38		1	1	2	100		46	6		6				1	2				
Lepocinclis texta	7-22-38	2	10	5		1				2	2	1								
" "	7-27-38	3	2	3	1	22			16		6									
Trachelomonas crebea	5-23-38	2	28	25	24	16			104	104	80	3	80	80	1	1	1	2		1
" "	8-19-38	30	40	120	40	40			90	60	40		100	4	10	6	1		3	
" urceolata	6-15-38	70	2	20	20	20		120	50	10	100		10		2				22	
" "	6-20-38	10		20	60	20		30	4	2	12		20		12	16	10			

Tributary Streams near junction with Scioto River

(a) Big Walnut
(b) Little Walnut
(c) Darby Creek
(d) Deer Creek
(e) Paint Creek
(f) Salt Creek

Euglenidae in Other Situations

If the green Euglenidae are not found predominately in the zones of highest pollution, what is their ecologic status relative to pollution? In June, 1938, a biological survey of the Licking River of Kentucky was made at a time when the river was clear and rather low. The main stream, rising in mountainous country, gets the sewage of a very small population, and flows rather swiftly until it meets the South Fork at Falmouth, Ky. The South Fork, much shorter, gets the sewage of several good sized towns - Paris, Cynthiana, Mt. Sterling, - but also has a much slower current, and has low head dams in three places. It is nowhere grossly polluted, and at Cynthiana is used as a water supply, although only a short distance below Paris. Examination at seven points on the North Fork, down almost to Falmouth, gave a total of six species of green Euglenidae of which only three, Euglena species, Trachelomonas urceolata, and T. volvocina, were sufficiently abundant to count; 2, 4 and 2 per ml. respectively. On the South Fork however, fifteen species were found, in numbers from 1 to 160 per ml. All of these were forms normally found in the Scioto, the most abundant being Euglena gracilis 160 per ml., Euglena species 100 per ml., Trachelomonas crebea, 90 per ml., T. urceolata, 160 per ml. and T. volvocina 30 per ml. These counts were made where there was no visible evidence of sewage pollution. The North Fork water was turbid with silt, and not aged greatly, due to rapid run off. The South Fork water was relatively clear, but possibly older water - and in addition, it had been polluted by sewage but not sufficiently to show visual evidence.

These two streams, the Scioto and Licking Rivers, present instances where there are large populations of green Euglenidae in areas below sewer outfalls. The maximum population of such plankton is not in grossly corrupted water, however, but below such stretches. The North Fork of the Licking also offers an instance of a moderately large and rapidly flowing stream which contains almost no sewage and has a small population of euglenid flagellates. A parallel case

is the Great Miami River and its tributary the Whitewater River. The latter is relatively swift and gets little sewage, whereas the Great Miami is slowed up by dams, gets the sewage and industrial wastes of several cities and at times has many Euglenidae. The Whitewater is less turbid than the North Fork of the Licking, and the Great Miami is comparatively clear but sometimes contains paper mill wastes.

These instances show that Euglenidae are certainly abundant in some waters containing sewage. It is our contention that clarity of water and age of water are factors favoring their development also, and that the pollution of a river by domestic sewage is not alone sufficient to cause the development of a large population of these green flagellates. Pollution, followed by ageing and clarifying of water may be more favorable to their development, and a seasonal factor may also be added. Pollution certainly seems to be a contributing factor, but to use these organisms as indicators of sewage contamination would require that sewage be more than a contributing factor for their excessive increase. In 1938, 48 samples were taken from the Cumberland and Duck Rivers in Tennessee. The upper watershed of the Cumberland is very rugged and run-off is fast, while the water at the time the samples were taken was decidedly turbid with erosion silt. In 183 miles of the Cumberland above mile 198 (from the mouth) the only Euglenidae found were Trachelomonas crebea and T. urceolata (figures 1 and 6) which occurred in 3 samples and a species of Euglena in one sample. Apparently, age of water, silt and lack of organic contamination militated against the organisms. At mile 218, the Cumberland begins to get material quantities of sewage; between mile 198 and 218 the sewered population numbers 26,000, and industrial wastes are poured into the river from two cheese plants, a condensed milk plant, a woolen mill and a large viscose rayon plant. At about mile 190, Nashville, population 150,000, empties its untreated sewage into the river. Sixteen miles below this point the greatest numbers of Euglenidae were found. While they were found in

all samples between miles 88 and 198, their total and average numbers were very low as compared with samples from the Scioto at the same time of year and only nine species were identified. One additional group termed Euglena sp. was present in small numbers, but the bulk of the population was made up of Trachelomonas crebea and T. urceolata whose distribution in the Scioto is quite variable. Euglena viridis occurred in small numbers in only 3 samples, not being found above mile 150. Evidently the addition of the sewage of a large city failed to be more than a very mild stimulus to the development of Euglenidae in the Cumberland River.

In September, 1939, the senior author again made a careful survey of the Cumberland, examining fresh samples taken from numerous stations above, at and below Nashville. The stream was very low, the temperature high, the flow was very sluggish and the stream clear. Despite these supposedly favorable conditions, the numbers of Euglenidae were few and there was no material increase at or below Nashville, although the polluted condition of the river was all too apparent.

With regard to the Duck River, the Tennessee Health Authorities (6) state that between miles 150 and 174 from the mouth, the river carries a heavy load of sewage pollution. Six known species of Euglenidae, and one unidentified (gracilis group?) Euglena were found in these samples in 1938 as follows:

River miles	98	122	130	136	186	216	225
Nos. of Euglenidae per ml.	26	80	106	436	38	29	4

Almost all of these were made up of Trachelomonas urceolata and Euglena sp. The large numbers at mile 136 are not due to sewage; to quote from Dr. Williams "this (sewage) pollution is not present at mile 136 since this point is above Columbia (Tenn.), and the nearest known pollution is that from Shelbyville, which is about 80 miles up stream." Actually, despite the sewage of Columbia, entering at mile 130, there is a steady decrease of these green flagellates downstream.

The Duck was also surveyed in September, 1939, and while a heterogenous plankton was present, there was no large population of Euglenidae anywhere in the

stream, regardless of the presence or absence of sewage. In short, biological surveys of these two streams have flatly failed to show that sewage encourages the growth of Euglenidae.

Discussion

Sudden enormous increases of microorganisms are well known to biologists, often with little or no indication of the cause. This is especially true of Euglenidae, and is shown perhaps in the numbers at Red Bridge (Fig. 3) in September and August, 1937 and 1938. An examination of the data for suspended solids, 5-day B.O.D., pH, 37° agar counts and coliform counts of bacteria shows no correlative aspect for the rise in August, the sudden drop on September 3, and the subsequent sharp rise and fall in 1937. The decline in early August, 1938, was due to a rain; either dilution of the volume of river water or the attendant jump in suspended solids (largely silt) from 160 p.p.m. to 530 p.p.m. There was a prompt recovery, however, to 790 individuals per ml. of raw river water, then another heavy rain about September 12, decreased numbers to about 25 per ml. The increases are very difficult to account for, and can hardly be attributed to changes in the river traceable to sewage. No known river factor - light, temperature, chemical composition, etc., undergoes such violent fluctuations as shown by figure 2, not even volume and turbidity following a rain.

The species listed for the Scioto are fairly well recognized. Only one group of the genus Euglena, comprising possibly Euglena agilis, E. gracilis and E. pisciformis offers much difficulty. Of these the senior author (7) has found only one, E. gracilis, abundant in sewage plants. Peterson (8) has reported E. polymorpha as abundant in this same plant. Euglena polymorpha has been noted (9) as a bloom on an unpolluted country pond, and we have also noted it as a bloom on water in a cedar swamp at Woods Hole, Mass. E. sanguinea we have found as a common late summer bloom on hot muddy ponds or borrow pits; Eddy (10) has reported it as a "bloom" on sink holes in Southern Illinois. E. mutabilis

has been found as a dominant (11) in highly acid coal mine drainage. E. deses we have procured in large numbers from stagnant, but not sewage polluted drainage ditches. E. oxyuris has been reported by Senior-White (12) as high as 100 per ml. in a "foul tank" used as a buffalo wallow at Delhi, India. E. viridis has constituted 98% of the population of a small pool fed by a small city sewer during street construction in Cincinnati. Eddy (13) reported a number of Euglenidae in the Sangamon River in 1929, but never as many as 1 per ml; he considers the river "a fairly clean stream." Kofoid (14) found many of these species in the Illinois River between 1894 and 1899 inclusive, when it was a clean stream, not getting the sewage of Chicago through the Drainage Canal. He recorded 24 species of this group, all but two of which have been found in the Scioto. Euglena viridis was most abundant, E. oxyuris next. None of the other abundant Scioto River Euglenidae were found in large numbers by him, but Trachelomonas volvocina occurred in the Illinois in abundance. In 1921-22 Purdy (15) reexamined the plankton of the Illinois and found only Euglena viridis in noteworthy numbers. Allen (16) found six species of Euglenidae in net samples from the San Joaquin River, but is is difficult to relate them to pollution. Such a search through the literature fails to show a definite correlation between sewage pollution and species, or even numbers, of Euglenidae present. Euglena viridis and E. oxyuris have been shown to be present in large numbers in polluted waters, but we question that they do not occur abundantly elsewhere. We believe that the finding of other species in great abundance where there is no evidence for sewage pollution shows the group as a whole should not be used as indicating sewage pollution. We do believe the evidence from the Scioto River studies indicates only one species, E. viridis, is valuable as such an indicator, and even it may occur in abundance in a zone where pollution is only revealed by careful chemical and bacteriological studies. Furthermore, of the Euglenidae as a whole, our experimental studies and a survey of the literature indicate that only the following species

tend to occur in abundance in sewage polluted water:

Cryptoglena pigra
Euglena fusca
" gracilis*
" oxyuris
" pisciformis
" polymorpha
" viridis
Lepocinclis texta
Phacus pyrum
Trachelomonas crebea
" urceolata"

*Probably includes several morphologically related species.

Since some of these have been found by us, and by other observers in huge numbers in environments not polluted by sewage, or in situations far from sewage pollution where recovery of a sick water was taking place, we believe that it is more accurate to state that the abundance of these organisms may mean pollution; probably means the presence of organic substances, or end products of decomposition of organic compounds. We do not believe they should be regarded as indicators of sewage pollution, unless other evidence is adduced in support.

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